

1 We claim:

- 2 1. A method of constructing a model of a biological network comprising steps of:  
3 providing a biological system or a plurality of biological systems, each  
4 biological system comprising a biological network comprising a plurality of  
5 biochemical species having activities;  
6 perturbing the activity of at least one of the biochemical species, thereby  
7 causing a response in the biological network;  
8 allowing the biological network to reach a steady state;  
9 determining the response of at least one of the biochemical species in the  
10 biological network; and  
11 estimating parameters of the model.
- 12 2. The method of claim 1, wherein the model comprises a set of differential  
13 equations or a set of difference equations that represent evolution over time of  
14 the activities of at least one of the biochemical species.
- 15 3. The method of claim 1, wherein the model comprises a polynomial  
16 approximation of a set of differential equations or a set of difference equations  
17 that represent evolution over time of the activities of at least one of the  
18 biochemical species.
- 19 4. The method of claim 1, wherein the model comprises a linear approximation of  
20 a set of differential equations or a set of difference equations that represent  
21 evolution over time of the activities of at least one of the biochemical species.
- 22 5. The method of claim 2, 3, or 4, wherein the differential or difference equations  
23 are nonlinear.
- 24 6. The method of claim 1, wherein determining the response of a biochemical  
25 species comprises measuring the activity of the biochemical species.

- 1     7.     The method of claim 1, wherein the response comprises an alteration in the  
2           activity of one or more of the biochemical species in the biological network or  
3           no alteration in any of the biochemical species.
- 4     8.     The method of claim 1, wherein the perturbing step comprises:  
5           applying a perturbation to a different biochemical species in the  
6           biological network in each of at least one of the biological systems, each  
7           biological system comprising a cell or a population of cells, and wherein the  
8           determining step comprises determining the response of at least one of the  
9           biochemical species in the biological network in each of at least one of the  
10          biological systems after allowing the biological network to reach a steady state.
- 11    9.     The method of claim 1, wherein the perturbing step comprises:  
12          applying a perturbation to one or more biochemical species in the  
13          biological network in each of at least one of the biological systems, each  
14          biological system comprising a cell or a population of cells, and wherein the  
15          determining step comprises determining the response of at least one of the  
16          biochemical species in the biological network in each of at least one of the  
17          biological systems after allowing the biological network to reach a steady state.
- 18    10.    The method of claim 8 or 9, wherein a single biochemical species in the  
19          biological network in each biological system is perturbed.
- 20    11.    The method of claim 8 or 9, wherein multiple biochemical species in each  
21          biological system are perturbed simultaneously.
- 22    12.    The method of claim 8 or 9, wherein each of the biochemical species in the  
23          biological network is perturbed in at least one of the biological systems.
- 24    13.    The method of claim 8 or 9, wherein each of the biological species in the  
25          biological network is perturbed in exactly one of the biological systems.
- 26    14.    The method of claim 8 or 9, wherein less than 100% of the biochemical species  
27          in the biological network are perturbed.

- 1    15.    The method of claim 1, wherein the perturbing step comprises:  
2                applying a perturbation to one or more biochemical species in the  
3                biological network in a biological system comprising a cell or a population of  
4                cells, and wherein the determining step comprises determining the response of  
5                at least one of the biochemical species in the biological network after allowing  
6                the biological network to reach a steady state; and  
7                repeating the applying and determining steps for each of at least one of  
8                the biochemical species in the biological network.
- 9    16.    The method of claim 15, wherein a single biochemical species in the biological  
10                network is perturbed in each applying step.
- 11   17.    The method of claim 15, wherein multiple biochemical species are perturbed  
12                simultaneously in each applying step.
- 13   18.    The method of claim 15, wherein each of the biochemical species in the  
14                biological network is perturbed in at least one of the applying steps.
- 15   19.    The method of claim 15, wherein each of the biological species in the biological  
16                network is perturbed in exactly one of the applying steps.
- 17   20.    The method of claim 15, wherein less than 100% of the biochemical species in  
18                the biological network are perturbed.
- 19   21.    The method of claim 1, wherein the biological system is a cell or a population  
20                of cells.
- 21   22.    The method of claim 21, wherein the cell or population of cells is prokaryotic or  
22                eukaryotic.
- 23   23.    The method of claim 1, wherein the activity of a biochemical species is its  
24                expression level.

- 1    24.    The method of claim 1, wherein the biochemical species comprise at least one  
2            of genes, deoxyribonucleic acids, ribonucleic acids, polypeptides, and  
3            metabolites, and wherein the activity of a biochemical species is its amount,  
4            concentration, maximum catalytic rate or expression level.
- 5    25.    The method of claim 1, wherein the step of estimating parameters comprises  
6            steps of:  
7                selecting a fitness function; and  
8                either computing the values of the parameters that optimize the fitness  
9            function; or  
10            (i) selecting a search procedure; and  
11            (ii) applying the selected search procedure so as to identify the values of  
12            the parameters that optimize the selected fitness function.
- 13   26.    The method of claim 25, wherein determining the response of a biochemical  
14            species comprises measuring the activity of the biochemical species, and  
15            wherein the fitness function compares measured values of the perturbations  
16            applied in the perturbing step with predictions of the measured values of the  
17            perturbations.
- 18   27.    The method of claim 26, wherein the predictions are obtained by using the  
19            measured activity values, selected values of the parameters, and the model to  
20            calculate values of the perturbations that would produce the measured activities,  
21            given the selected values of the parameters and the model.
- 22   28.    The method of claim 25, wherein the fitness function is selected from the group  
23            consisting of: a minimum total square error function, a maximum square error  
24            function, and a total absolute error function.
- 25   29.    The method of claim 25, wherein the search procedure is selected from the  
26            group consisting of: a Simplex algorithm, a gradient descent algorithm, and a  
27            simulated annealing algorithm.

- 1    30.    The method of claim 25, further comprising the step of:  
2                    imposing a constraint on the biological network so as to limit values that  
3                    may be used to optimize the fitness function.
- 4    31.    The method of claim 30, wherein the search procedure is selected from the  
5                    group consisting of: a Simplex algorithm, a gradient descent algorithm, and a  
6                    simulated annealing algorithm.
- 7    32.    The method of claim 30, wherein the constraint comprises one or more  
8                    constraints selected from the group consisting of: (i) fixing the number of  
9                    regulatory inputs to each biochemical species; (ii) minimizing the number of  
10                   non-zero parameters; (iii) restricting parameters to discrete values; and (iv)  
11                   requiring parameters that provide a dynamically stable model.
- 12   33.    The method of claim 32, further comprising the step of:  
13                    estimating the number of regulatory inputs to each biochemical species,  
14                    wherein the constraint comprises fixing the number of regulatory inputs to each  
15                    biochemical species.
- 16   34.    The method of claim 32, wherein the constraint comprises selecting the  
17                    maximum number of regulatory inputs to each biochemical species and,  
18                    wherein the search procedure comprises:  
19                    (a) generating all putative network structures including one or more  
20                    regulatory inputs per biochemical species, but not more regulatory inputs than  
21                    the maximum number of regulatory inputs;  
22                    (b) calculating or searching for parameters that optimize a chosen fitness  
23                    function for each putative network structure; and  
24                    (c) selecting as a solution whichever of the putative networks of step (b),  
25                    comprising a network structure and parameters, optimizes the fitness function.
- 26   35.    The method of claim 34, wherein the fitness function compares measured values  
27                    for perturbations applied in the perturbing step with predictions of the measured  
28                    values of the perturbations.

- 1    36.    The method of claim 35, wherein the predictions are obtained by using the  
2            measured activity values, selected values of the parameters, and the model to  
3            calculate values of the perturbations that would produce the measured activities,  
4            given the selected values of the parameters and the model.
- 5    37.    The method of claim 32, wherein the constraint comprises fixing the number of  
6            regulatory inputs to each biochemical species; and wherein the search procedure  
7            comprises steps of:  
8                (a) generating one or more putative network structures including one or  
9                more input connections per biochemical species;  
10              (b) calculating or searching for parameters that optimize a chosen fitness  
11              function for each putative network structure;  
12              (c) selecting one or more of the networks of step (b), each putative  
13              network comprising a structure and parameters, with optimal fitness as  
14              determined by the fitness function;  
15              (d) determining whether any of the putative networks selected in part (c)  
16              satisfies some chosen stop criterion;  
17              (e) if the stop criterion is met, stopping the search and selecting one or  
18              more putative network structures and parameters; and  
19              (f) if the stop criterion is not met, generating one or more variants of the  
20              network structures selected in step (c) and returning to step (b).
- 21    38.    The method of claim 37, wherein the fitness function compares measured values  
22            for perturbations applied in the perturbing step with predictions of the measured  
23            values of the perturbations.
- 24    39.    The method of claim 38, wherein the predictions are obtained by using the  
25            measured activity values, selected values of the parameters, and the model to  
26            calculate values of the perturbations that would produce the measured activities,  
27            given the selected values of the parameters and the model.

- 1 40. The method of claim 37, wherein the searching in step (b) is performed using a  
2 search procedure selected from the group consisting of: a Simplex algorithm, a  
3 gradient descent algorithm, and a simulated annealing algorithm.
- 4 41. The method of claim 37, wherein the search procedure is selected from the  
5 group consisting of: Forw-reest-*K*, Forw-Top*D*-reest-*K*, Forw-Float-*K*, Back-*K*,  
6 Back-reest-*K*, Genalg-SteadyState-*K*, Genalg-Gen-*K*, and Exhaustive-*K*.
- 7 42. The method of claim 37, wherein the stop criterion requires that the putative  
8 network attains a predetermined level of fitness, that the putative network  
9 comprises a selected number of regulatory inputs, or that the change in the level  
10 of fitness between subsequent iterations of the steps (b) and (c) is less than a  
11 predetermined amount.
- 12 43. The method of claim 32, wherein the constraint comprises minimizing the  
13 number of nonzero parameters.
- 14 44. The method of claim 43, wherein the fitness function is the total square error  
15 function and the search procedure identifies auxiliary parameters that minimize  
16 a function consisting of the sum of (i) the minimum length vector of the  
17 parameters that minimize the total square error fitness function and (ii) the  
18 product of (a) the auxiliary parameters and (b) a matrix of vectors spanning the  
19 nullspace of a matrix comprising the measured activities.
- 20 45. The method of claim 1, wherein the estimated parameters are considered  
21 random variables; and comprising the additional step of estimating the  
22 probability density function for each estimated parameter.
- 23 46. The method of claim 45, wherein estimating the probability density function for  
24 each estimated parameter comprises estimating one or more of the first, second,  
25 third or higher moments of the probability density function.
- 26 47. The method of claim 46, wherein one or more of the first, second, third or  
27 higher moments of the probability density function for each parameter are

- 1       estimated using the measured activity values and the measured perturbation  
2       values.
- 3   48.   The method of claim 47, wherein the estimated first and second moments of the  
4       probability density functions of the estimated parameters are used to calculate  
5       the statistical significance of the one or more of the estimated parameters.
- 6   49.   The method of claim 48, wherein the statistical significance of one or more of  
7       the estimated parameters are calculated using one or more tests selected from  
8       the group consisting of the z-test, the t-test, and the chi-squared-test.
- 9   50.   A method of performing sensitivity analysis on a biological network comprising  
10       steps of:  
11       generating or providing a model of the biological network according to  
12       the method of claim 1; and  
13       determining the sensitivity of the activities of a first set of one or more  
14       species in the network to a change in the activities of a second set of one or  
15       more species in the network using the model.
- 16   51.   The method of claim 50, further comprising the step of:  
17       identifying the second set of species as a major regulator of the first set  
18       of species if the sensitivity of the first set of species to a change in the activities  
19       of the second set of species meets a predefined criterion.
- 20   52.   The method of claim 50, wherein the determining step comprises:  
21       inverting a matrix of parameters of the model, wherein the parameters  
22       quantify regulatory relationships between species in the model, thereby  
23       obtaining a gain matrix, wherein each column or row of the gain matrix  
24       represents the sensitivity of species in the model to a perturbation of one of the  
25       species.
- 26   53.   The method of claim 50, wherein the sensitivity of the activities a first set of  
27       biochemical species to a change in the activities of a second set of biochemical



- 1 species is a measure of the change in activities of the first set of species in  
2 response to a change in activities of the second set of species.
- 3 54. The method of claim 53, wherein the measure is a quantitative measure.
- 4 55. The method of claim 54, wherein the quantitative measure is the mean  
5 percentage change in activities of the first set of species in response to a unit  
6 change in activities of the second set of species.
- 7 56. The method of claim 51, wherein the predefined criterion is a requirement that  
8 sensitivity of the activities of at least one species in the first set of species to a  
9 change in the activities of the second set of activities is statistically different  
10 from zero.
- 11 57. The method of claim 51, wherein the predefined criterion is a requirement that  
12 the sensitivity of the activities of at least one species in the first set of species to  
13 a change in the activities of the second set of activities exceeds a predetermined  
14 value.
- 15 58. The method of claim 51, wherein the predefined criterion is a requirement that  
16 the sensitivity of the activities of the first set of species to a change in the  
17 activities of the second set of species is greater than the sensitivity of the first  
18 set of species to a change in the activities of a third set of one or more species.
- 19 59. A method of identifying a target of a perturbation comprising steps of:  
20 providing a biological system comprising a biological network  
21 comprising a plurality of biochemical species having activities;  
22 providing or generating a model of the biological system constructed  
23 according to the method of claim 1;  
24 perturbing one or more biochemical species in the network;  
25 allowing the biological network to reach a steady state;  
26 determining the response of at least one of the biochemical species in the  
27 biological network to the compound; and

1                   calculating predicted perturbations of biochemical species in the  
2                   biological network that would be expected to yield the determined responses  
3                   according to the model.

4   60.   The method of claim 59, wherein the perturbing step comprises contacting the  
5           biological system with a compound, thereby causing a response in the biological  
6           network, and wherein the target is a target of the compound.

7   61.   The method of claim 59, further comprising the step of:  
8                   identifying a biochemical species as a target of the perturbation if the  
9           predicted perturbation to that biochemical species meets a predefined criterion.

10   62.   The method of claim 61, wherein the predefined criterion is a requirement that  
11           the strength of the predicted perturbation to the biochemical species exceeds a  
12           predetermined value.

13   63.   The method of claim 61, wherein the predefined criterion is a requirement that  
14           the strength of the predicted perturbation is identified as statistically significant.

15   64.   The method of claim 63, wherein a predicted perturbation is identified as  
16           statistically significant by using a statistical test selected from the group  
17           consisting of the z-test, the t-test, and the chi-squared-test.

18   65.   The method of claim 64, wherein the statistical test is used with estimates of the  
19           first and second moments of the probability density functions of the predicted  
20           perturbations, wherein the estimates of the first and second moments are  
21           calculated from measured values of the responses of the biochemical species  
22           and measured values of the perturbations applied in the perturbing step.

23   66.   A method for identifying phenotypic mediators comprising steps of:  
24                   comparing parameters of models of biological networks for a plurality of  
25           biological systems, wherein the models are generated according to the method  
26           of claim 1, and wherein the biological networks comprise overlapping or  
27           substantially identical sets of biochemical species; and

1 identifying biochemical species for which associated parameters differ  
2 between the models as candidate phenotypic mediators.

3 67. The method of claim 66, wherein one or more of the biological systems display  
4 differences in one or more properties.

5 68. The method of claim 66, wherein the properties include the steady-state  
6 activities of the biochemical species of the biological system, the phenotype of  
7 the biological system, and the genotype of the biological system.

8 69. The method of claim 66, wherein a species is identified as a phenotypic  
9 mediator if the difference between the parameters for that species in some or all  
10 of the models satisfies a predefined criterion.

11 70. The method of claim 69, wherein the predefined criterion is a requirement that  
12 the difference exceeds a predefined value.

13 71. The method of claim 69, wherein the predefined criterion is a requirement that  
14 the difference achieves a particular level of statistical significance.

15 72. A computer system for constructing a model of a biological network, the  
16 computer system comprising:  
17 memory that stores a program comprising computer-executable process  
18 steps; and  
19 a processor which executes the process steps so as to construct a model  
20 of a biological network, the model comprising an approximation to a set of  
21 differential equations or a set of difference equations that represent evolution  
22 over time of activities of at least one biochemical species in a biological  
23 network.

24 73. The computer system of claim 72, wherein the process steps estimate  
25 parameters of and select a structure for a model of a biological network.

26 74. The computer system of claim 73, wherein the process steps comprise steps of:

- 1                   (a) selecting or receiving a selection of a fitness function; and  
2                   (b) either computing the values of the parameters that optimize the  
3 fitness function; or  
4                   (i) selecting or receiving a selection of a search procedure; and  
5                   (ii) applying the search procedure so as to identify the values of  
6 the parameters that optimize the fitness function.

- 7 75. The computer system of claim 74, wherein the search procedure comprises:  
8                   (a) generating all putative network structures including one or more  
9 regulatory inputs per biochemical species, but not more regulatory inputs than  
10 the maximum number of regulatory inputs;  
11                   (b) calculating or searching for parameters that optimize a chosen fitness  
12 function for each putative network structure; and  
13                   (c) selecting as a solution whichever of the putative networks of step (b),  
14 comprising a network structure and parameters, optimizes the fitness function.

- 15 76. A computer system for performing sensitivity analysis of a biological network,  
16 the computer system comprising:  
17                   memory that stores a program comprising computer-executable process  
18 steps; and  
19                   a processor which executes the process steps so as to  
20                   (i) generate or receive a model of a biological network, the model  
21 comprising an approximation to a set of differential equations or a set of  
22 difference equations that represent evolution over time of activities of at least  
23 one biochemical species in a biological network; and  
24                   (ii) determine the sensitivity of the activities of a first set of one or more  
25 species in the network to a change in the activities of a second set of one or  
26 more species in the network using the model.

- 27 77. A computer system for identifying a target of a perturbation comprising  
28                   memory that stores a program comprising computer-executable process  
29 steps; and

1 a processor which executes the process steps so as to  
2 (i) construct or receive a model of a biological network, the model  
3 comprising an approximation to a set of differential equations or a set of  
4 difference equations that represent evolution over time of activities of at least  
5 one biochemical species in a biological network; and  
6 (ii) receive data comprising responses of at least one of the biochemical  
7 species to the perturbation; and  
8 (iii) calculate predicted perturbations of biochemical species in the  
9 biological network that would be expected to yield the determined responses  
10 according to the model.

11 78. A computer system for identifying a phenotypic mediator, the computer system  
12 comprising:

13 memory that stores a program comprising computer-executable process  
14 steps; and

15 a processor which executes the process steps so as to

16 (i) compare parameters of models of biological networks for a plurality  
17 of biological systems, wherein the models comprise an approximation to a set of  
18 differential equations or a set of difference equations that represent evolution  
19 over time of activities of at least one biochemical species in a biological  
20 network, and wherein the biological networks comprise overlapping or  
21 substantially identical sets of biochemical species; and

22 (ii) identify one or more biochemical species for which associated  
23 parameters differ between the models as candidate phenotypic mediators.

24 79. Computer-executable process steps stored on a computer-readable medium, the  
25 computer-executable process steps to construct a model of a biological network,  
26 the computer-executable process steps comprising:

27 code to construct a model of a biological network, the model comprising  
28 an approximation to a set of differential equations or a set of difference

- 1 equations that represent evolution over time of activities of at least one  
2 biochemical species in a biological network.
- 3 80. Computer-executable process steps as set forth in claim 79, wherein the code  
4 comprises code to estimate parameters of and select a structure for a model of a  
5 biological network.
- 6 81. Computer-executable process steps as set forth in claim 80, wherein the code to  
7 estimate parameters and select a structure comprises  
8 code to select or receive a selection of a fitness function; and  
9 code to perform either or both of (a) and (b), where (a) and (b) are:  
10 (a) code to compute the values of the parameters that optimize the  
11 fitness function;  
12 (b) code to  
13 (i) select or receive a selection of a search procedure; and  
14 (ii) apply the selected search procedure so as to identify the  
15 values of the parameters that optimize the selected fitness function.
- 16 82. Computer-executable process steps as set forth in claim 80, wherein the search  
17 procedure comprises steps of:  
18 (a) generating all putative network structures including one or more  
19 regulatory inputs per biochemical species, but not more regulatory inputs than  
20 the maximum number of regulatory inputs;  
21 (b) calculating or searching for parameters that optimize a chosen fitness  
22 function for each putative network structure; and  
23 (c) selecting as a solution whichever of the putative networks of step (b),  
24 comprising a network structure and parameters, optimizes the fitness function.
- 25 83. Computer-executable process steps stored on a computer-readable medium, the  
26 computer-executable process steps to perform sensitivity analysis of a  
27 biological network, the computer-executable process steps comprising:

- 1 (i) code to generate or receive a model of a biological network, the  
2 model comprising an approximation to a set of differential equations or a set of  
3 difference equations that represent evolution over time of activities of at least  
4 one biochemical species in a biological network; and  
5 (ii) code to determine the sensitivity of the activities of a first set of one  
6 or more species in the network to a change in the activities of a second set of  
7 one or more species in the network using the model.

8 84. Computer-executable process steps stored on a computer-readable medium, the  
9 computer-executable process steps to identify a target of a perturbation, the  
10 computer-executable process steps comprising:

- 11 (i) code to estimate parameters of and select a structure for a model of a  
12 biological network or to receive estimated parameters of and a structure for a  
13 model of a biological network, the model comprising an approximation to a set  
14 of differential equations or a set of difference equations that represent evolution  
15 over time of activities of at least one biochemical species in a biological  
16 network;  
17 (ii) code to receive data comprising responses of at least one of the  
18 biochemical species to the perturbation; and  
19 (iii) code to calculate predicted perturbations of biochemical species in  
20 the biological network that would be expected to yield the determined responses  
21 according to the model.

22 85. Computer-executable process steps stored on a computer-readable medium, the  
23 computer-executable process steps to identify a phenotypic mediator, the  
24 computer-executable process steps comprising:

- 25 (i) code to compare parameters of models of biological networks for a  
26 plurality of biological systems, wherein the models comprise an approximation  
27 to a set of differential equations or a set of difference equations that represent  
28 evolution over time of activities of at least one biochemical species in a

1 biological network, and wherein the biological networks comprise overlapping  
2 or substantially identical sets of biochemical species; and  
3 (ii) code to identify one or more biochemical species for which  
4 associated parameters differ between the models as candidate phenotypic  
5 mediators.  
6